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MINIREVIEW

Extracellular Enzymes with Immunomodulating Activities: Variations on a Theme in *Streptococcus pyogenes*

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In order to successfully colonize or invade a host, microorganisms need mechanisms to escape recognition by the immune system or to modulate the responses directed against the infecting agent. *Streptococcus pyogenes* produces a number of extracellular enzymes, several of which interact with the host immune system. These interactions might be of importance for the host-parasite interplay and the development of disease. Of specific interest are enzymatic activities that are targeted toward components of the host immune system (summarized in Table 1). These enzymes either directly or indirectly modulate the activity of immune defense molecules such as immunoglobulins, complement factors, or other inflammatory mediators. Our review presents an update on immunomodulating enzymes from *S. pyogenes* and discusses their influence on the development of both acute and secondary diseases. These enzymatic activities are also discussed in a wider perspective by comparisons with similar systems of other human pathogens.

S. pyogenes is one of the most common human pathogens and the causative agent of pharyngitis accounting for 15 to 30% of all cases in children and 5 to 10% in adults (5). *S. pyogenes* also infects skin and soft tissue, especially among people living in warm and humid climates. Most of these infections, such as impetigo, erysipelas, and cellulitis, are localized to the skin (6). However, in a significant proportion of these infections bacteria disseminate into deeper tissue, which subsequently leads to necrotizing fasciitis with substantial destruction of fascia and adipose tissue. Further dissemination of the bacteria can ultimately lead to sepsis and a toxic shock syndrome (TSS) with high mortality. The incidence of these types of serious infection has increased lately, with an overrepresentation of isolates of the M1 and M3 serotypes (82, 102).

In addition to acute infections, there are a number of aseptic sequelae affecting different organ systems. For example, acute poststreptococcal glomerulonephritis (APSGN) that can lead to renal failure, and acute rheumatic fever (ARF) presenting with joint inflammation, carditis, symptoms from the central nervous system, and skin manifestations (reference 21 and references therein).

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MICROBIAL IMMUNOGLOBULIN PROTEASES

Immunoglobulins (antibodies) produced by B lymphocytes in response to foreign material are crucial molecules in the humoral and mucosal defense against infectious agents. Antibodies that are directed toward microorganisms recruit complement factors and direct leukocytes to the site of infection, which ultimately leads to phagocytosis and killing of the microorganism. In order to combat an attack from the immune system many microbial pathogens produces enzymes that cleave or inactivate immunoglobulins, which have been suggested to contribute to pathogenesis. For instance, microbial proteases capable of cleaving the hinge region of human mucosal antibodies, e.g., immunoglobulin A (IgA), have been extensively studied. Even though the flexible hinge region of IgA1 is protected from proteolysis by multiple O-linked glycans (75), several pathogens have evolved specific IgA-proteases that cleave at specific sites in the hinge region of IgA and thus overcome the protective ability of the glycans (for a review, see reference 84). The first examples of IgA-proteases were described in *Streptococcus sanguis* and *Neisseria* spp. in the mid-1970s (85). Subsequently, IgA-proteases have been described for a number of bacterial species that colonize or infect the mucosal membranes of humans, such as oral streptococci (57), *Haemophilus influenzae*, and *Streptococcus pneumoniae* (58, 72). As a result of this specific IgA-protease activity, the IgA molecule is cleaved into a stable Fc fragment and two monomeric Fab fragments that retain their antigen-binding capacity (73, 74). IgA2 is more resistant to proteolysis due to the lack of a specific peptide stretch that can be found in the hinge region of IgA1 (87). These IgA-proteases have been shown to inactivate IgA by cleaving in the hinge region (86), but their importance as virulence determinants has been debated. Early studies suggested that IgA-protease activity distinguishes pathogenic from nonpathogenic *Neisseria* spp. (80), and recent studies indicate that invasive *Neisseria meningitidis* isolates are enhanced in the IgA-protease activity compared to colonizing strains (110). Furthermore, IgA-proteases have been identified as virulence factors in nontypeable *H. influenzae* infections (109).

Interestingly, no specific IgA-protease that cleaves the hinge region has been described in *S. pyogenes*. However, it was recently shown that the activity from the streptococcal cysteine proteinase SpeB is capable of degrading the COOH-terminal part of IgA (15). The importance of this IgA-degrading activity needs to be further investigated (see below).

TABLE 1. Immunomodulating enzymatic activities expressed by *S. pyogenes*

Enzyme	Type of enzyme	Activities	Reference(s)
C5a peptidase	Serine proteinase	Cleaves the complement factor C5a	114
EndoS	Endo-β-*N*-acetylglucosaminidase	Hydrolyzes the N-linked glycan on IgG	16
IdeS/Mac	Cysteine proteinase	Cleaves IgG and inhibits opsonophagocytosis	61, 111
NADase	NAD^+-glycohydrolase ADP-ribosyltransferase	Alters neutrophil migration Translocates into cells mediated by SLO	55, 71, 103
SAGP	Arginine deiminase	Antitumor activity; inhibition of T-cell proliferation	23, 24, 52
SOD	SOD	Detoxification of oxygen radicals	35
SpeB	Cysteine proteinase	See Table 2	See Table 2

Microbial IgG-proteases have not been as extensively studied as the IgA-proteases. However, there are accumulating data describing microbial IgG-degrading proteases. These include the oral pathogens *Prevotella intermedia* and *Prevotella nigrescens* isolated from periodontal pockets and oral abscesses. Inhibition experiments suggest that these pathogens degrade IgG due to cysteine proteinase activity (47). In addition, a *Pseudomonas aeruginosa* elastase implicated as a virulence factor degrades human IgG in vitro, and its activity could be inhibited by local treatment with the protease inhibitor α_2-macroglobulin (43). Furthermore, a secreted cysteine proteinase from the helminth parasite *Paragonimus westermani* attenuated the effector functions of human eosinophils stimulated with IgG (100), and proteases from *Serratia marcescens* cleave both IgG and IgA around the hinge region (78). Thus, modulations of IgG by cysteine proteinases seem to be a common theme among pathogenic microorganisms.

Streptococcal cysteine proteinase SpeB. *S. pyogenes* expresses a cysteine proteinase, SpeB, shown to be identical to the streptococcal pyrogenic-erytrogenic exotoxin B (33, 38). This proteinase is one of the most extensively studied secreted proteins from *S. pyogenes.* Its activity on the streptococcal M protein and human fibrin was discovered by Stuart Elliott already in the 1940s (28). The gene encoding SpeB is highly conserved and is present in essentially all *S. pyogenes* isolates (117). The three-dimensional structure of the zymogen form of SpeB has been determined, revealing a fold similar to papain despite negligible sequence identity (51). Papain is a cysteine proteinase that cleaves IgG in the hinge region and is commonly used to obtain Fc and monomeric Fab fragments from IgG (see Fig. 1A) (8).

Interestingly, it was recently shown that SpeB cleaves IgG in a manner similar to IgA-proteases; IgG is cleaved at a defined site in the hinge region into two stable monomeric Fab fragments and one Fc fragment (16). The degradation pattern of IgG resembles that of papain, but the cleavage site is different; SpeB cleaves between two glycines where the IgG heavy chain is more flexible (Fig. 1A). It is not very surprising that the IgG

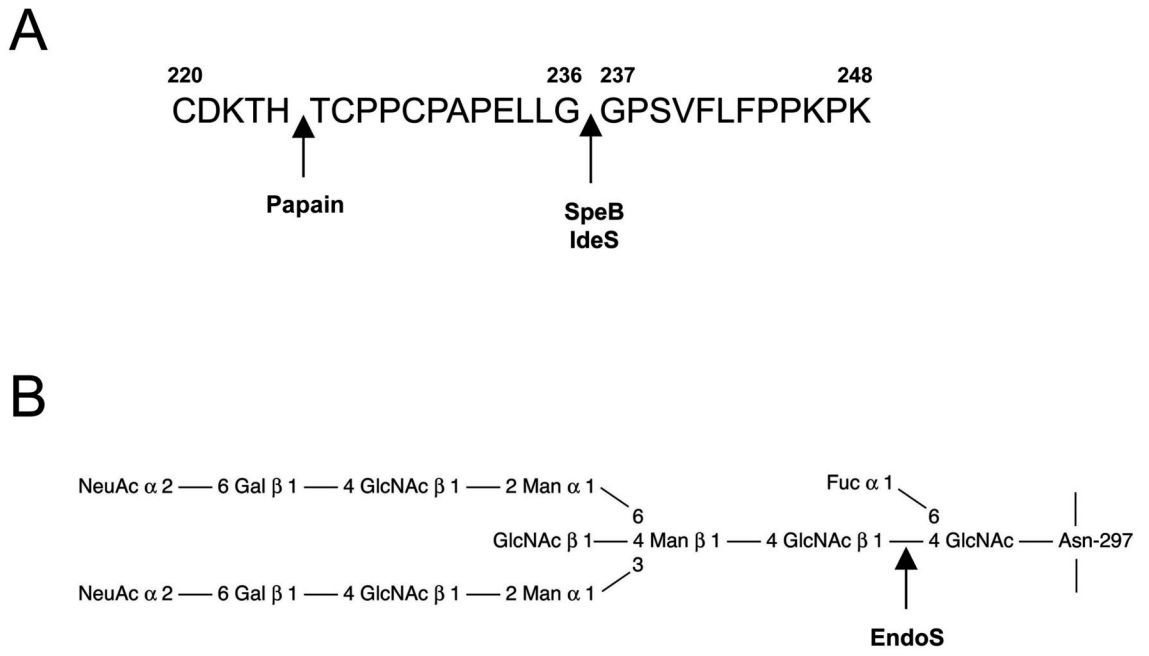

FIG. 1. SpeB, IdeS, and EndoS activity on human IgG. Cleavage sites in human IgG of *S. pyogenes* IgG-proteases and IgG glycan-hydrolases. (A) Residues 220 to 248 of the flexible hinge region of IgG1 heavy chain showing where SpeB and IdeS cleave between glycine residues 236 and 237. The papain cleavage site is shown for comparison. (B) Diagram showing one of the two identical conserved N-linked glycans attached to asparagine 297 in the heavy chain of IgG and also where EndoS hydrolyzes the chitibiose core of the glycan, leaving the innermost GlcNAc with a core fucose.

TABLE 2. Immunomodulating activities of SpeB

Host molecule	Activity	Reference
H-kininogen	Release of bradykinin	42
IL-1β	Activation	54
Decorin	Release of dermatan sulfate that inhibits antibacterial peptides	97
Antibacterial peptide LL-37	Degradation	96
IgG	Cleavage in the hinge region	16
IgA, IgM, IgD, and IgE	Degradation	15
Histamine	Release from mast cells	113

degradation by SpeB significantly reduces the capacity of opsonizing IgG to kill *S. pyogenes* in human blood (18). This was further supported when an isogenic SpeB mutant strain was shown to persist a significantly shorter time than the corresponding wild-type strain in blood containing strain-specific antibodies (29). SpeB was the first described *S. pyogenes* enzyme with immunoglobulin-protease activity, adding to the growing list of its activities, emphasizing its involvement in pathogenesis. In addition to the activity on IgG, SpeB degrades the COOH-terminal parts of the heavy chains of IgA, IgM, and IgD into low-molecular-weight fragments, whereas the heavy chains of IgE are completely degraded (15).

SpeB also has other immunomodulating activities such as the release of proinflammatory molecules that could be important for the symptoms seen in *S. pyogenes* infections (summarized in Table 2). For instance, the cytokine precursor interleukin-1β (IL-1β) is cleaved by SpeB into an active IL-1β, which is a strong inflammatory mediator (54). Furthermore, SpeB has the ability to release the potent proinflammatory and vasoactive peptide hormone bradykinin from its precursor H-kininogen (42). This release of bradykinin could be one of the explanations for the hypovolemic hypotension seen in sepsis caused by *S. pyogenes*. Interestingly, kinin release by cysteine proteinases has also been demonstrated in the periodontitis-causing bacterium *Porphyromonas gingivalis* (98), as well as in the parasitic protozoan *Trypanosoma cruzi* (25). In addition, SpeB can degrade proteoglycans such as decorin with the release of dermatan sulfate that inhibits the neutrophil-derived antibacterial peptide α-defensin (97). Furthermore, SpeB directly cleaves and inactivates the antibacterial peptide LL-37, which is capable of killing *S. pyogenes* (96). Moreover, a recent study showed that purified SpeB stimulates the release of histamine from a human mast cell line (113). Some reports have suggested that SpeB also functions as a superantigen, with stimulation of T lymphocytes without antigen presentation, and that the activity is independent of the proteolytic activity (30, 64). In contrast, evidence has been presented that SpeB does not have superantigenic properties and that the observed activity rather originates from contaminating SpeA, SpeC, or unknown mitogens (34).

The regulation of SpeB activity is highly complex and influenced by a number of parameters during synthesis (summarized in Fig. 2). SpeB is transcribed during early stationary phase and downregulated by glucose and other nutrients in the growth medium (11). The global transcriptional regulator *mga* (91), the *speB*-specific *ropB* in the Rop loci (regulation of proteinase) (70), and *pel* (pleotropic effect locus) have been shown to be positive regulators of *speB* expression (65). Whether the two-component system CsrR-CsrS represses *speB* is still debated (31, 40). Two peptide permeases have also been suggested to regulate SpeB production (88, 89).

The proregion of SpeB has a unique fold and inactivation mechanism that displaces the catalytically essential His residue from the active site (51). It is known that purified zymogen from streptococci is partly enzymatically active and can cleave itself under reducing conditions (9). Autocatalysis is an intermolecular event with sequential processing with at least six intermediates (26). This process does not occur when *ropA*, the second gene of the Rop loci, has been inactivated. RopA assists SpeB in translocation via the secretory pathway and functions as a molecular chaperone that aids the zymogen to its autocatalytically active state (70). Furthermore, when there is no cell wall-anchored M protein, the SpeB zymogen is secreted in a conformational state that does not allow autocatalytical processing (17).

Active SpeB is inhibited by a peptide inhibitor based on the active site of cystatin C (7). *S. pyogenes* binds the broad-spec-

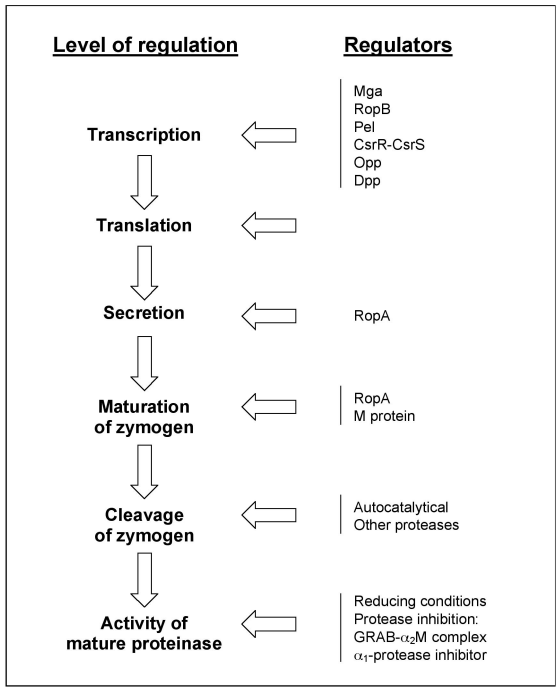

FIG. 2. Schematic overview of the factors influencing SpeB activity. The column to the left outlines the synthesis and maturation of SpeB from transcription to mature active enzyme. The right column shows examples of different regulators know to affect the process and on what level it takes place.

trum proteinase inhibitor α_2-macroglobulin via the cell wall-anchored protein GRAB, forming a complex that regulates proteolytic activity of SpeB (94). The S-nitrosylated form of α_1-protease inhibitor also inhibits SpeB (76).

SpeB production does not affect bacterial viability in vitro (10), but many animal studies have suggested its importance for the balance of the host-parasite interaction. For example, isogenic *speB* mutant strains are significantly less lethal to mice when challenged intraperitoneally (68) and caused less mortality and tissue damage when mice were infected subcutaneously (59, 67). Furthermore, bacteria lacking SpeB are less resistant to phagocytosis and do not disseminate into internal organs as do the wild-type bacteria (66). SpeB also plays a role in host tissue tropism, since SpeB activity increased the bacterial reproduction in a mouse impetigo model (104), and SpeB acts synergistically with cell wall antigens and streptolysin O (SLO) to induce lung injury in rats (99).

The relevance of animal models can be debated, especially since *S. pyogenes* exclusively infects humans, but there are some studies of human infections also supporting a role for SpeB in pathogenesis, even though there are somewhat conflicting results. Patients with invasive disease caused by different serotypes of *S. pyogenes* seroconverted to SpeB, indicating that SpeB is expressed in vivo during infection (36). On the other hand, patients with severe invasive disease have low antibody titers against SpeB, suggesting that an inability to produce SpeB-specific antibodies contributes to the development of serious conditions (44). Furthermore, isolates of the M1 serotype from TSS patients are associated with SpeB production (105). In contrast, another study showed that there is an inverse relationship between SpeB production and disease severity, possibly due to a sparing of the M protein on the surface (53). Epidemiological evidence suggests a correlation between SpeB production and a genetic marker for preferred tissue site of infection at the skin (104). Furthermore, SpeB has been suggested to play a role in the development of APSGN (20); patients with APSGN have elevated antibody levels against SpeB, and SpeB can be detected in glomerulonephritis biopsies (19).

Taken together, SpeB is a multifunctional protease with several immunomodulating activities that could affect both mucosal and systemic immunological functions. Even though there are conflicting reports of the importance of SpeB as a virulence factor, it is clear that SpeB needs to be taken into account in any consideration of the various aspects of the interaction between *S. pyogenes* and the human host both during acute infection and in aseptic sequelae.

IdeS, a second cysteine proteinase. In addition to SpeB, a second secreted cysteine proteinase, IdeS (immunoglobulin G-degrading enzyme of *S. pyogenes*), was recently discovered (111). Interestingly, IdeS cleaves human IgG in the hinge region at the same position as SpeB (Fig. 1A). This results in an inhibition of antibody-mediated phagocytosis of the bacteria. IdeS, which has a higher degree of specificity compared to SpeB, does not degrade the other immunoglobulin isotypes (111). Interestingly, IdeS is identical to the previously described protein Mac (group A streptococcal Mac-1-like protein) that binds Fc receptors (61). Lei et al. (61) suggested that the activity of Mac/IdeS mimics that of a leukocyte integrin, Mac-1, and thereby inhibits opsonophagocytosis of the bacteria. However, von Pawel-Rammingen et al. (111) discovered that IdeS is a cysteine proteinase that specifically cleaves IgG in the hinge region, which most likely explains the activity described for Mac. Furthermore, an enzymatically inactive IdeS generated by site-directed mutagenesis still binds to neutrophils but does not inhibit phagocytosis as the wild-type protein (112). Whether IdeS/Mac can inhibit phagocytosis independently of proteolytic activity is still debated (62), and a recent study reported that site-directed mutagenesis of the active site did not affect the ability to inhibit phagocytosis (63). The gene encoding IdeS was shown to be present in many isolates (111), and the protein is secreted by several serotypes. Moreover, patients with *S. pyogenes* infections have antibody titers against IdeS, indicating in vivo expression (61). Interestingly, a recent study identified a protein identical to IdeS/Mac as a surface-localized anchorless protein that binds with high affinity to immunoglobulins, thus confirming the interaction with IgG, but did not discuss the cysteine proteinase activity or similarity to IdeS/Mac (56). Taken together, these data indicate that *S. pyogenes* expresses two unrelated cysteine proteinases that can cleave IgG, thus emphasizing the importance of this immunomodulating mechanism.

IMMUNOGLOBULIN GLYCAN-HYDROLASES

Apart from enzymes capable of cleaving or degrading the peptide backbone of IgG, *S. pyogenes* expresses enzymes that hydrolyze the conserved N-linked glycans on glycoproteins. First, an extracellular neuraminidase activity was described to release sialic acid from bovine submaxillary mucins (22, 39). Another study showed that strains isolated from patients with APSGN produce a neuraminidase activity that releases terminal sialic acids from the glycans on human IgM, IgG, fibrinogen, and renal basement membranes. These alterations of the immunoglobulins were suggested to play a role in the development of APSGN, since all of the nephritogenic strains tested, but none of the rheumatogenic strains tested, expressed this activity (79). However, no neuraminidase gene has been described or found in the *S. pyogenes* genomes that have been sequenced, and convincing data indicate that *S. pyogenes* does not produce any true neuraminidase (95). The sialic acid-releasing activity previously observed is most likely due to other glycan-hydrolyzing enzymes, such as EndoS, that cleave further down in the N-linked glycans of human glycoproteins.

EndoS, a specific IgG glycan-hydrolase. An extracellular endoglycosidase, EndoS, which has a specific activity on the conserved N-linked glycan located in the constant portion of the heavy chain of IgG, was recently identified (16). EndoS is a 108-kDa secreted enzyme with a conserved family 18-chitinase motif. Enzymes belonging to this family hydrolyze *N*-acetylglucosamine polymers (chitin) and some of them, e.g., EndoF and EndoH, have activity on the chitin core of N-linked glycans on glycoproteins (41, 107, 108). EndoS hydrolyzes the β1,4-di-*N*-acetylchitobiose core on the N-linked oligosaccharide of IgG, which leaves the innermost GlcNAc with an attached fucose on the peptide backbone (Fig. 1B).

The conserved N-linked glycan on the IgG heavy chain is crucial for several effector functions, including complement activation and binding to Fc receptors on leukocytes (69, 93, 115). Therefore, a bacterial enzyme hydrolyzing this function-

ally important glycan could have profound effects on IgG-mediated processes.

A classical method in streptococcal research was developed by Lancefield (60) that involves a bactericidal or antiphagocytic assay based on the notion that *S. pyogenes* with M protein present on its surface survives in fresh human nonimmune blood but is rapidly opsonized and killed if the blood contains M-type-specific antibodies.

An experiment was designed to investigate the importance of the conserved glycan structure on opsonizing antibodies. Thus, when purified opsonizing IgG directed against the M protein was treated with EndoS in vitro and used in a modified classical bactericidal assay, it was significantly impaired in its ability to kill *S. pyogenes* bacteria in human blood compared to untreated IgG (18). The main explanations for the reduced killing of bacteria was the inability of the EndoS-treated IgG to bind to Fc receptors on monocytic cells and also decreased IgG-mediated complement deposition. Our results underline the functional importance of the glycan on IgG and reveal a novel interaction between pathogenic bacteria and immunoglobulins. Thus, EndoS is an example of a bacterial strategy that interferes with the function of an important host defense molecule, IgG. The specificity of EndoS was further reinforced when native and denatured IgG was incubated with EndoS, which revealed that when IgG is denatured, EndoS is unable to hydrolyze the glycan (15). As a comparison, many other endoglycosidases such as $EndoF_1$ and $EndoF_2$ have enhanced activities when the substrate glycoprotein is denatured (106). It indicates that the tertiary structure of the whole IgG molecule, and not only the glycan, is important for the enzymatic activity.

EndoS was originally identified in the AP1 strain of M1 serotype by N-terminal sequencing of extracellular proteins. By using the first completed *S. pyogenes* genome-sequencing project of the M1 strain SF370, it was possible to identify the complete gene encoding EndoS, *ndoS*, which was subsequently sequenced also in the AP1 strain (16, 32) (accession numbers NP_269818 and AAK00850, respectively). PCR experiments revealed that the *ndoS* gene could be amplified in strains of 10 different M serotypes, suggesting that *ndoS* is a widely distributed in *S. pyogenes* (M. Collin and A. Olsén, unpublished results). In addition, *ndoS* homologs have been sequenced and annotated in the M18 strain MGAS8232 completed sequencing project (101) (accession number AAL98385), as well as in the recently published genome of the M3 strain MGAS315 (3) (accession number AAM80175). Furthermore, a similar open reading frame could be identified in the unfinished genome sequence of the *S. pyogenes* M5 strain Manfredo (http://www.sanger.ac.uk/Projects/S_pyogenes/).

Interestingly, an open reading frame similar to *ndoS* could also be identified in the unfinished genome sequence of a *Streptococcus equi* strain (http://www.sanger.ac.uk/Projects/S_equi/). *S. equi* belongs to group C streptococci and is closely related to group A streptococci (*S. pyogenes*). *S. equi* is primarily a horse pathogen, but group C streptococci can cause a number of suppurative mucosal infections in mammals, including humans (37). Preliminary experiments indicate that group C streptococci isolated from human infections secrete an EndoS homolog that hydrolyzes the glycan on human IgG, which cross-react with EndoS antibodies (Collin and Olsén, unpublished).

When the deduced amino acid sequences of the proteins similar to EndoS are aligned, it is clear that the proteins are highly similar and most likely true homologs (Fig. 3). The sequence data are still limited, but this might indicate a selective pressure on streptococci infecting humans to conserve these IgG glycan hydrolases.

Immunoglobulin glycosylation and autoimmunity. Abnormalities in IgG glycosylation have been discovered in a number of human autoimmune disorders such as systemic lupus erythematosus, inflammatory bowel diseases, and rheumatoid arthritis (27, 83). In rheumatoid arthritis, it has been demonstrated that isolated B lymphocytes have reduced galactosyltransferase activity, leading to higher levels of truncated, so-called agalactosyl glycans on IgG (2). Defects in IgG-glycosylation have also been shown to be important for the development of cryoglobulin-induced lupus-like glomerulonephritis in mice (77). An interesting hypothesis could thus be postulated: that IgG hydrolyzed by EndoS is important in the development of ARF and APSGN, where autoimmunity and antibody complexes are implicated in the disease processes. It should be noted that one of the major factors shown to contribute to the development of APSGN are the specific streptokinases (45, 81), but of potential interest is the finding that a neuraminidase activity against IgG is associated with glomerulonephritis induced by *S. pyogenes* (79). As previously discussed, EndoS might contribute to the observed neuraminidase activity, but further investigation is needed to evaluate the importance of EndoS in the development of diseases such as ARF and APSGN

COMPLEMENT-DEGRADING ENZYMES

C5a peptidase. One of the most studied immunomodulating enzymes from *S. pyogenes* is the C5a peptidase, ScpA. ScpA is an excellent example of an enzyme that targets a specific component of the human immune defense. ScpA is a cell-wall-anchored 130-kDa serine endopeptidase that specifically cleaves the complement factor C5a (14, 114). By cleaving the chemotactic complement factor C5a, ScpA inhibits recruitment of phagocytic cells to the infectious site (49). C5a has also been shown to be important in activating neutrophils that phagocytize the bacteria, underlining the relevance of the activity of ScpA (50). Furthermore, intranasal immunization with C5a peptidase has been shown to prevent nasopharyngeal colonization of mice by *S. pyogenes* (48). Moreover, in a mouse model of long-term colonization, an *S. pyogenes* strain lacking the gene encoding ScpA caused pneumonia at a lower frequency than did wild-type bacteria (46). An interesting finding is that SpeB can release functional fragments of ScpA that subsequently inactivate C5a at a distance from the bacterium (4). The *scpA* gene has been shown to be highly similar to the group B streptococcal *scpB* gene, suggesting horizontal gene transfer between the species (13). Furthermore, ScpB in group B streptococci has been shown to contribute to cellular invasion in addition to the enzymatic activity (12). Taken together, these findings indicate that ScpA is important for the disease process of *S. pyogenes* infections.

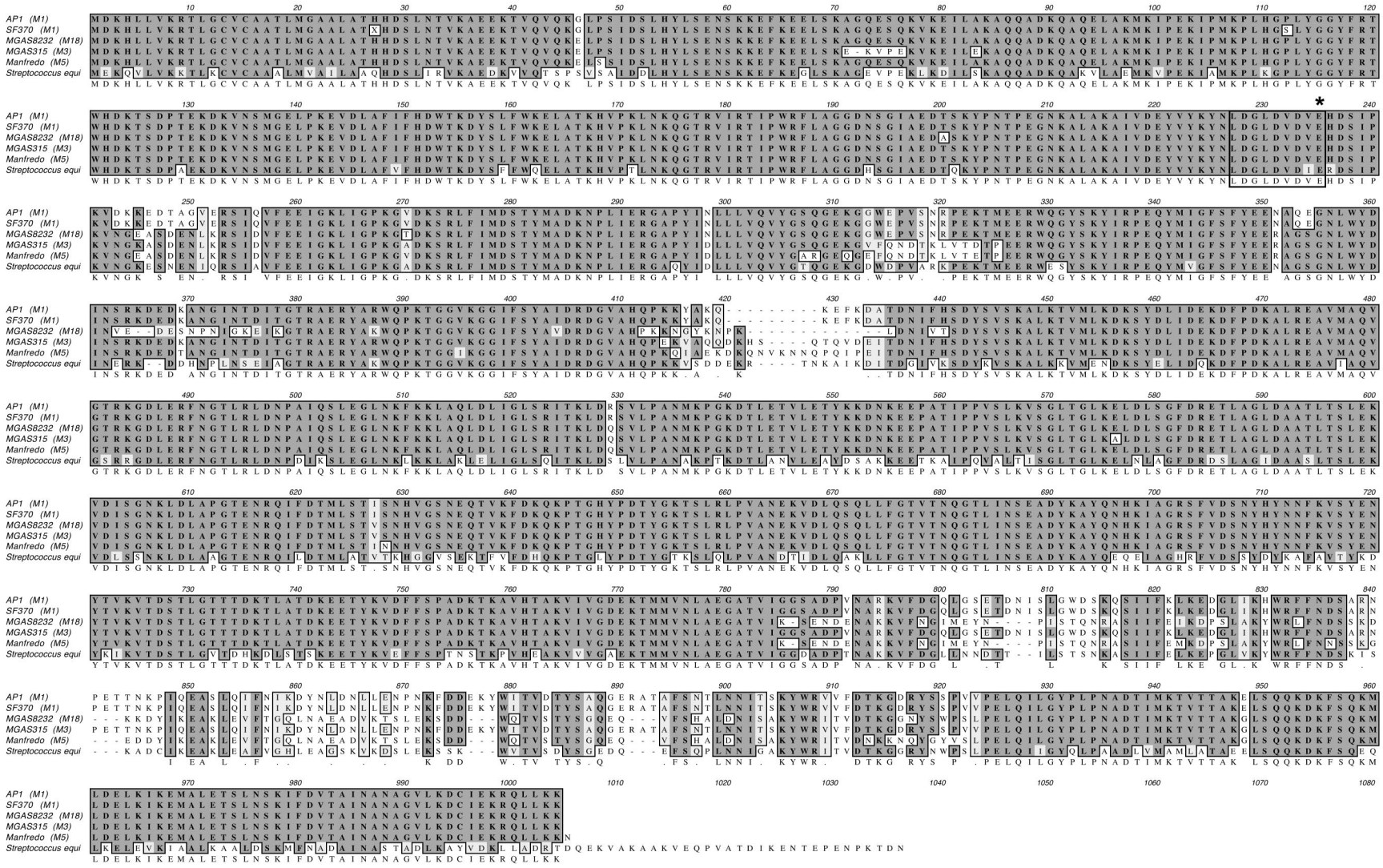

FIG. 3. CLUSTALW amino acid sequence alignment of EndoS homologs from different *S. pyogenes* serotypes and *S. equi*. Strain names or species are shown to the left and M serotypes are shown in parentheses. Amino acid identities are shown in dark gray, similarities are shown in light gray, and the consensus sequence is shown under the alignment. The conserved chitinase motif is boxed, and the glutamic acid essential for activity is marked with an asterisk above the alignment.

ADDITIONAL ENZYMES WITH IMMUNOMODULATING ACTIVITIES

In addition to the immunomodulating activities such as protein hydrolysis or glycan modification, *S. pyogenes* produces various enzymes belonging to other classes. One such enzyme is represented by the streptococcal acid glycoprotein (SAGP) that was originally described as an antitumour protein (52). SAGP has arginine deiminase activity and inhibits T-lymphocyte proliferation (24). In addition, this SAGP is important for bacterial survival at low pH, possibly contributing to intracellular survival (23).

Another enzyme is the NAD^+-glycohydrolase (NADase) that not only hydrolyzes NAD^+ into adenosin-diphosphoribose and nicotinamide but also synthesizes the signaling molecule cyclic ADP-ribose (1, 55). It has also been shown that the pore-forming cytolysin SLO aids NADase to penetrate the membrane of host cells and thereby induces cytotoxicity (71). An explanation for the earlier finding could be that NADase purified from group A streptococci alters neutrophil-directed migration (103). Moreover, in their study they also found NADase to be expressed by *S. pyogenes* strains associated with an outburst of TSS, suggesting that NADase activity contributes to the development of severe streptococcal disease (103).

Superoxide anions are involved in the phagocytic killing of bacteria. Bacterial superoxide dismutases (SODs) detoxify superoxide anions and are a major defense mechanism against oxidative stress. Genes encoding SOD have been identified in several gram-positive bacteria, including *S. pyogenes* (92). *S. pyogenes* produces a manganese-dependent SOD (35), but no direct evidence for a role of SOD in oxidative stress resistance has been presented. However, insertional inactivation of *sodA*, encoding a manganese-dependent SOD homolog in *S. pneumoniae*, significantly reduced the pneumococcal virulence in a mouse model (116). This indirectly suggests that SOD in *S. pyogenes* could be of importance for resistance against oxygen radicals produced by the hosts phagocytic cells.

CONCLUDING REMARKS

The strictly human pathogen *S. pyogenes* has evolved a number of extracellular enzymes of various types that interact with the host immune defense. Some of them, like SpeB, are nonspecific and targeted toward many host molecules, whereas others, such as the C5a peptidase, EndoS, and IdeS, are specific for single molecules in the human immune system. Thus, *S. pyogenes* expresses a set of broad-spectrum detoxifying and immunomodulating enzymes, providing basal protection against, for instance, oxygen radicals and low pH, and also a number of specific immunomodulating enzymes targeted toward important host molecules, such as IgG and the complement factor C5a. This indicates that *S. pyogenes* has evolved several different enzymatic strategies to circumvent host defense mechanisms in order to successfully colonize and disseminate within a host. Moreover, the fact that one of the key molecules in the adaptive immune response, IgG, serves as a substrate for at least two proteases (SpeB and IdeS) and one endoglycosidase (EndoS) is unlikely to be a coincidence but rather indicates a underlying evolutionary pressure driving the development of enzymes modulating immunoglobulins and also conservation of such genes. This hypothesis is supported by the fact that the *speB* gene is present in most isolates and is, in addition, highly conserved. Even though there is still limited sequence data available for *ndoS* and *ideS*, these genes seem to be abundant and/or highly conserved as well.

One could argue that a hydrolyzing activity toward immunoglobulins, which circulate in high concentrations in human blood, could primarily have evolved for bacterial nutrient acquisition. However, when it comes to SpeB it has been shown that its regulation is influenced by nutrient concentrations but is not directly involved in the acquisition of the nutrients (90). Furthermore, an isogenic EndoS mutant strain was not affected in its growth rate compared to the wild type when it was grown in a plasma environment, suggesting that EndoS does not play a major role in nutrient acquisition (16).

Some of the discussed enzymatic activities inhibit inflammatory processes (immunoglobulin and complement degradation and inhibition of T-lymphocyte proliferation), whereas others are proinflammatory (kinin and interleukin release, superantigenic activity). Probably all of these activities working in different directions contribute to balancing the host-parasite relationship, leading to a more successful colonization and spread of the bacteria. Unbalanced or uncontrolled production of enzymes could contribute to severe invasive disease but also be of importance for postinfectious autoimmune reactions such as ARF and APSGN. Further studies on extracellular enzymes targeted toward components of the immune defense will broaden our understanding of how this pathogen interacts with its host and causes disease.

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